ORIGINAL SUBMISSION

000001

RECEIVED
MAR 30 2004
OFFICE OF
FOOD ADDITIVE SAFETY

novozymes®

March 26, 2004

Linda S. Kahl, Ph.D.
Office of Food Additive Safety, HFS-255
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Dr. Kahl,

We are hereby submitting, in triplicate, a generally recognized as safe (GRAS) notification, in accordance with proposed 21 C.F.R. § 170.36, for Novozymes' glucanase enzyme preparation produced by *Trichoderma harzianum*. The glucanase enzyme preparation is intended for use in the wine industry.

Please contact me by direct telephone at 919 494-3151 or direct fax at 919 494-3420 if you have any questions or require additional information.

Sincerely,

Lori Gregg
Regulatory Specialist

Enclosures (3 binders)

Novozymes North America, Inc.
77 Perry Chapel Church Road
P.O. Box 576
Franklinton, North Carolina 27525

Tel: 919-494-3000

Fax: 919-494-3450

Internet: www.novozymes.com

000002

RECEIVED
MAR 30 2004
OFFICE OF
FOOD ADDITIVE SAFETY

March 26, 2004

novozymes®

RE: GRAS Notification - Exemption Claim

Dear Sir or Madam:

Pursuant to the proposed 21C.F.R.§ 170.36 (c)(1) Novozymes North America Inc. hereby claims that glucanase preparations produced by submerged fermentation of *Trichoderma harzianum* are Generally Recognized as Safe; therefore, they are exempt from statutory premarket approval requirements.

The following information is provided in accordance with the proposed regulation:

Proposed § 170.36 (c)(1)(i) *The name and address of the notifier.*

Novozymes North America Inc.
77 Perry Chapel Church Rd., Box 576
Franklinton, NC 27525

Proposed § 170.36 (c)(1)(ii) *The common or usual name of notified substance.*

Glucanase enzyme preparation from *Trichoderma harzianum.*

Proposed § 170.36 (c)(1)(iii) *Applicable conditions of use.*

The glucanase is intended for use in the wine industry to improve clarification and filtration of wines, especially those wines produced from *Botrytis*-infected grapes. The enzyme preparation is used at minimum levels necessary to achieve the desired effect and according to requirements for normal production following Good Manufacturing Practices.

Proposed §170.36 (c)(1)(iv) *Basis for GRAS determination.*

This GRAS determination is based on scientific procedures.

Proposed § 170.36 (c)(1)(v) *Availability of information.*

A notification package providing a summary of the information which supports this GRAS determination is enclosed with this letter. The package includes a safety evaluation of the production strain, the enzyme, and the manufacturing process, as well as an evaluation of dietary exposure. Complete data and information that are the basis for this GRAS determination are available to the Food and Drug Administration for review and copying upon request.

Date

Director, Regulatory Affairs

Novozymes North America, Inc.
77 Perry Chapel Church Road
P.O. Box 576
Franklinton, North Carolina 27525

Tel: 919-494-3000 *Fax: 919-494-3450* *Internet: www.novozymes.com*

000003

novozymes®

A glucanase preparation produced by

Trichoderma harzianum

Lori Gregg, Regulatory Affairs, Novozymes North America Inc., USA
Birger Rostgård Jensen, Regulatory Affairs, Novozymes A/S, Denmark

March 2004

000004

9086S4

novozymes®

TABLE OF CONTENTS

000005

novozymes®

1. GENERAL INTRODUCTION

The subject of this notification is a glucanase preparation (Novozymes A/S trade name Glucanex® 200 G) produced by submerged fermentation of a *Trichoderma harzianum* strain. Glucanase is a multicomponent enzyme preparation that contains an exo-β-glucanase activity (EC 3.2.1.58, glucan 1,3-β-glucosidase), which has utility in the wine industry.

The glucanase enzyme preparation is used to improve the clarification and filtration of wines, especially those wines produced from *Botrytis*-infected grapes. The enzyme preparation selectively degrades the beta-1,3-1,6-glucan polysaccharide produced in the grapes by the fungus *Botrytis cinerea* or by yeast.

The information provided in the following sections is the basis for our determination of general recognition of safety of the glucanase enzyme preparation produced by *T. harzianum*. The safety of the production organism must be the prime consideration in assessing the probable degree of safety of an enzyme preparation intended for use in food [1,2]. The production organism for this glucanase, *T. harzianum*, is discussed in Sections 2 and 7.

Our safety evaluation in Section 7 includes a discussion of the evaluation of the production strain, the enzyme, and the manufacturing process, as well as an evaluation of dietary exposure to the preparation.

2. PRODUCTION MICROORGANISM

2.1 Production Strain

The production microorganism, *T. harzianum,* belongs to the Hyphomycetes (imperfect fungi). Strains of this species are widely used in research and industrial biotechnology as producers of industrial enzymes. They have also received attention as a potential biocontrol agent of plant disease due to their mycoparasitic properties[3].

T. harzianum is not considered a pathogenic microorganism[3] and is not closely related to any known pathogenic fungi. An extensive review of the literature shows that this species has only once been reported as a cause of an opportunistic infection in a patient with severe underlying disease[4].

JECFA specifications[9] state that "the evaluation of fungal sources for toxigenicity shall include a determination that they do not produce toxicologically significant amounts of mycotoxins that are known to be synthesized by members of the production organism's species or closely related species". While *T. harzianum* is

known as a producer of a wide variety of secondary metabolites, there have been no published reports connecting isolates of *T. harzianum* or any other closely related species with production of secondary metabolites with known toxicity against humans or animals. Glucanex® 200 G product was tested for the presence of the mycotoxin T-2 reported to be produced by *Trichoderma* sp. and none was found.

The production strain used to make the glucanase enzyme preparation has not been modified using recombinant DNA techniques.

2.2 Absence of Production Organism in Product

The absence of the production organism is an established specification for the commercial product, and as such, the production organism does not end up in the food.

3. MANUFACTURING PROCESS

This section describes the manufacturing process for the glucanase derived from *T. harzianum*, which follows standard industry practices[5-7]. The quality management system used in the manufacturing process for glucanase complies with the requirements of ISO 9001. The enzyme preparation is also manufactured in accordance with current good manufacturing practices (cGMP).

3.1 Raw Materials

The raw materials used in the fermentation and recovery process for the glucanase enzyme concentrate are standard ingredients used in the enzyme industry[5-7]. The raw materials conform to *Food Chemicals Codex* (FCC)[8] specifications except those raw materials that do not appear in the FCC. For those not appearing in the FCC, internal specifications have been made in line with FCC requirements. On arrival at Novozymes A/S, the raw materials are sampled by the Quality Control Department and subjected to the appropriate analyses to ensure their conformance to specifications.

The antifoams used in fermentation and recovery are used in accordance with the Enzyme Technical Association submission to FDA on antifoams and flocculants dated April 10, 1998. The maximum use level of these antifoams in the glucanase product is less than 1%.

3.2 Fermentation Process

The glucanase enzyme preparation is manufactured by submerged fed-batch pure culture fermentation of a *T. harzianum* strain. All equipment is carefully

designed, constructed, operated, cleaned, and maintained so as to prevent contamination by foreign microorganisms. During all steps of fermentation, physical and chemical control measures are taken and microbiological analyses are done to ensure absence of foreign microorganisms and confirm strain identity.

3.2.1 Production Organism

Each batch of the fermentation process is initiated with a lyophilized stock culture of the production organism, *T. harzianum,* described in Section 2. Each new batch of the stock culture is thoroughly controlled for identity, absence of foreign microorganisms, and enzyme-generating ability before use.

3.2.2 Criteria for the Rejection of Fermentation Batches

Growth characteristics during fermentation are observed both macroscopically and microscopically. Samples are taken from both the seed fermentor and the main fermentor before inoculation, at regular intervals during cultivation, and before transfer/harvest. These samples are tested for microbiological contamination by microscopy and by plating on a nutrient agar followed by a 24-48 hour incubation period.

The fermentation is declared "contaminated" if one of the following conditions is fulfilled:

1. Infection is observed in 2 or more samples by microscopy

2. Infection is observed in two successive agar plates at a minimum interval of 6 hours

Any contaminated fermentation is rejected.

3.3 Recovery Process

The recovery process is a multi-step operation, which starts immediately after the fermentation process and consists of both the purification and the formulation processes.

3.3.1 Purification Process

The enzyme is recovered from the culture broth by the following series of operations:

1. Pretreatment - pH adjustment

2. Primary Separation - vacuum drum filtration

3. Pre- and Germ Filtration - for removal of residual production strain organisms and as a general precaution against microbial degradation

4. Concentration - ultrafiltration and/or evaporation

5. Pre- and Germ Filtration - for removal of residual production strain organisms and as a general precaution against microbial degradation

3.3.2 Formulation and Standardization Processes

The liquid concentrate is spray dried on to a maltodextrin carrier by means of atomization into a fluidized spray dryer. The product is discharged batch wise after sieving. The product is standardized to the declared enzyme activity by addition of maltodextrin.

3.4 Quality Control of Finished Product

The final products are analyzed according to the specifications given in section 5.

4. ENZYME IDENTITY

Key enzyme and protein chemical characterizations of the glucanase are given below:

Classification:	Glucanase (generic name)
IUB nomenclature:	Glucan 1,3-β-glucosidase (common name)
IUB No.:	3.2.1.58
CAS No.:	9073-49-8
Reaction:	Successive hydrolysis of β-D-glucose units from the nonreducing ends of 1,3-β-D-glucans, releasing D-glucose

5. COMPOSITION AND SPECIFCATIONS

The glucanase enzyme preparation is presently available in a formula for use in wine applications.

5.1 Quantitative Composition

Glucanex® 200 G has the following typical composition:

Maltodextrin	approx.	82%
Enzyme concentrate, dry matter	approx.	13 %
Water	approx.	3 %
Citric acid	approx.	1%
Sodium citrate, tri, dihydrate	approx.	1%

5.2 Specifications

The glucanase enzyme preparation conforms to the general and additional requirements for enzyme preparations as described in *Food Chemicals Codex*[8]. In addition, the glucanase also conforms to the General Specifications for Enzyme Preparations Used in Food Processing as proposed by the Joint FAO/WHO Expert Committee on Food Additives in Compendium of Food Additive Specifications[9].

The following Novozymes' specifications have been established for the glucanase:

Enzyme activity	according to declaration
Heavy metals	not more than 30 ppm
Lead	not more than 5 ppm
Arsenic	not more than 3 ppm
Total viable count/g	not more than 1×10^4
Total coliforms/g	not more than 30
Enteropathogenic *E. coli*/25 g	negative by test
Salmonella/25 g	negative by test
Antibiotic activity	negative by test
Production organism	negative by test
Mycotoxins	negative by test

Heavy metals, lead, arsenic, antibiotic activity, and mycotoxins are analyzed at regular intervals.

Glucanex® 200 G has a typical activity of 200 BGXU/g. One Botrytis β-glucan unit (BGXU) is the amount of enzyme that releases 1 mmol reducing carbohydrate (calculated as glucose) per minute under the given standard conditions.

6. APPLICATION

6.1 Mode of Action

The enzyme of use to the wine industry in Glucanex® 200 G is glucanase, which catalyzes the hydrolysis of β-D-glucose units from the nonreducing ends of 1,3-β-D-glucans, releasing D-glucose. In winery applications, the enzyme preparation is used to improve clarification and filterability of wines made from *Botrytis*-infected grapes. The enzyme preparation selectively degrades the beta-1,3-1,6-glucan polysaccharide produced in the grapes by the fungus *Botrytis cinerea* or by yeast. The use of glucanase in wine clarification and filtration has been published[10].

A Product Sheet for this enzyme is enclosed as Appendix 1.

6.2 Use Levels

The enzyme preparation is used at minimum levels necessary to achieve the desired effect and according to requirements for normal production following cGMP.

In the wine industry, the recommended dosage of Glucanex® 200 G is 1-3 g/hl or 38-114 g/1000 gal.

6.3 Enzyme Residues in the Final Food

The enzyme preparation is added to the wine after the alcoholic fermentation in order to benefit from the higher temperature, but before any other fining steps prior to bottling. Depending on the fining agents used, the enzyme may or may not be removed from the final food.

7. SAFETY EVALUATION

7.1 Safety of the Production Strain

T. harzianum is not considered a pathogenic microorganism[3] and is not closely related to any known pathogenic fungi. An extensive review of the literature shows that this species has only once been reported as a cause of an opportunistic infection in a patient with severe underlying disease[4]. Furthermore, *T. harzianum* has not been classified in any of the known hazard classifications.

While *T. harzianum* is known as a producer of a wide variety of secondary metabolites, there have been no published reports connecting isolates of *T. harzianum* or any other closely related species with production of secondary

metabolites with known toxicity against humans or animals. In a recent report, Blumenthal makes recommendations for appropriate mycotoxin testing for enzyme preparations derived from certain fungal species. The author recommends that, for *Trichoderma*-derived enzyme preparations, a test for the trichothecene T-2 toxin be made[11]. The Glucanex® 200 G enzyme preparation was found to be negative for T-2 toxin.

The safety of the production organism must be the prime consideration in assessing the probable degree of safety of an enzyme preparation intended for use in food[2]. If the organism is nontoxigenic and nonpathogenic, then it is assumed that food or food ingredients produced from the organism, using current Good Manufacturing Practices (cGMP), is safe to consume[3]. Pariza and Foster (1983) define a nontoxigenic organism as "one which does not produce injurious substances at levels that are detectable or demonstrably harmful under ordinary conditions of use or exposure" and a nonpathogenic organism as "one that is very unlikely to produce disease under ordinary circumstances". *T. harzianum* meets these criteria for nontoxigenicity and nonpathogenicity.

7.2 Safety of the Glucanase Enzyme

Enzyme proteins themselves do not generally raise safety concerns[2,12,13]. As indicated in section 4, the subject enzyme of this notification is a glucanase, IUB EC 3.2.1.58, which causes the hydrolysis of β-D-glucose units from the nonreducing ends of 1,3-β-D-glucans, releasing D-glucose.

Glucanase enzyme preparations have a long history of safe use as processing aids in the food industry[2]. Developed in the 1980s, the glucanase from *T. harzianum* has been approved for use in wine production in the European Union since 1992[14]. The glucanase enzyme preparation has been evaluated during several Joint FAO/WHO Expert Committee on Food Additives (JECFA) meetings[15,16]. The Committee determined that "potential intake would be minimal if the enzyme was used as specified in the production of wine" and that "it had been provided with sufficient information to establish an ADI 'not specified' for the enzyme when used in accordance with good manufacturing practices in winemaking".

7.3 Safety of the Manufacturing Process

The glucanase from *T. harzianum* meets the general and additional requirements for enzyme preparations as outlined in the monograph on Enzyme Preparations in the *Food Chemicals Codex*[8]. As described in Section 3, the glucanase preparation is produced in accordance with current good manufacturing practices, using ingredients that are acceptable for general use in foods, and under conditions that ensure a controlled fermentation. These methods are

based on generally available and accepted methods used for production of microbial enzymes[5-7].

7.4 Safety Studies

This section describes the studies and analyses performed to evaluate the safety of the use of the glucanase enzyme preparation.

7.4.1 Description of Test Material

The safety studies were conducted on a liquid glucanase enzyme concentrate that was prepared according to the description given in Section 3, except that stabilization and standardization were omitted, as described by Elvig and Pedersen[17].

7.4.2 Studies

The following studies were performed:

-13 weeks subchronic oral toxicity in rats
-Test for mutagenic activity (Ames test)
-Chromosome aberration assay in mammalian cells

The results of these studies have been published[17]; a copy of the publication is provided as Appendix 2.

7.5 Estimates of Human Consumption and Safety Margin

As stated in Section 6.3, the glucanase enzyme may be removed from the wine during fining, however, in order to illustrate a "worst case" situation, the following calculation is made assuming that all enzyme protein is retained in the wine product.

The maximum recommended dosage of glucanase in wine production is 9 BGXU/L. The highest reported annual per capita consumption of wine in 1997 was for Luxembourg at 63.3 L[18,19].

The daily consumption for a person weighing 60 kg results in a maximum estimated daily intake (EDI) of:

$$9 \times (63.3/365)/60 = 0.026 \text{ BGXU/kg body weight (kgbw)/day.}$$

The enzyme test material used in the 13-week subchronic oral toxicity study contained 12.1 g Total Organic Solids (TOS) and 18,100 BGXU per 100 g. This equates to 0.668 mg TOS per 1 BGXU, thus the EDI TOS would be:

0.01736 mg TOS/kgbw/day

The no-observed-adverse-effect level (NOAEL) of 10 mL/kgbw/day in the 13-week subchronic oral toxicity study corresponds to an overall intake of:

1257.3 mg TOS/kgbw/day.

The safety margin would therefore be:

NOAEL/EDI = 1257.3 / 0.01736 = 72,425 or 7.2×10^4.

7.6 Results and Conclusion

The results of the tests described in Section 7.4.2 show that the glucanase enzyme preparation does not exhibit any mutagenic activity or toxic effect under the conditions of each specific test. On the basis of the safety evaluations contained in Sections 7.1-7.5, a review of the literature, and the history of safe use of *T. harzianum*, the glucanase enzyme preparation can be safely manufactured and used as a processing aid in the wine industry as well as in other food or non-food applications.

8. LIST OF APPENDICES

1. Novozymes Product Sheet for Glucanex® 200 G

2. Elvig, S. G. and P. B. Pedersen. 2003. Safety evaluation of a glucanase preparation intended for use in food including a subchronic study in rats and mutagenicity studies. Reg. Toxicol. and Pharm. 37:11-19.

9. REFERENCES

1. Pariza, M.W. and Johnson, E.A. Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. Reg. Toxicol. Pharm. 33:173-186, 2001.

2. Pariza, M.W. and Foster, E.M. Determining the safety of enzymes used in food processing. J. Food Protection, 46:5:453-468, 1983.

3. Samuels, G. J. *Trichoderma*–a review of biology and systematics of the genus. Mycological Research 100(8):923-935, 1996.

4. Guiserix, J. M. et al. *Trichoderma harzianum* peritonitis in peritoneal dialysis. Nephron 74:473-474, 1996.

5. Enzyme Applications in Encyclopedia of Chemical Technology, 4th ed., Ed. Kroschwitz, J.I., Volume 9, pp. 567-620, 1994.

6. Aunstrup, K., Andersen, O., Falch, E.A., and Nielsen, T.K.. Production of Microbial Enzymes in Microbial Technology, 2nd ed., Vol. I, Eds. Peppler, H.J. and Perlman, D., Chapter 9, pp.282-309, 1979.

7. Aunstrup, K.. Production, Isolation, and Economics of Extracellular Enzymes in Applied Biochemistry and Bioengineering, Volume 2, Enzyme Technology, Eds. Wingard, L.B., Katchalski-Katzir, E. And Goldstein, L, pp. 28-68, 1979.

8. National Academy of Sciences, Food and Nutrition Board, Committee on Food Chemicals Codex, Food Chemicals Codex, 5th Edition, The National Academies Press, Washington, D.C., p. 151, 2003.

9. JECFA (Joint FAO/WHO Expert Committee on Food Additives), General Specifications and Considerations for Enzyme Preparations Used in Food Processing, Compendium of Food Additive Specifications, FAO FNP (Food and Nutrition Paper) 52, Add.9, FAO, Rome, 2001.

10. Villettaz, J-C., D. Steiner, and H. Trogus. The use of a beta-glucanase as an enzyme in wine clarification and Filtration. Am. J. Enol. Vitic. 35(4):253-256, 1984.

11. Blumenthal, Cynthia. Production of toxic metabolites in *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*. Reg. Toxicol. Pharm. 2004, in print.

12. Food and Drug Administration. Lipase Enzyme Preparation From *Rhizopus niveus*: Affirmation of GRAS status as a Direct Food Ingredient. Fed. Regist. 63:24416-24419, 1998.

13. Food and Drug Administration. Statement of Policy: Foods Derived From New Plant Varieties. Fed. Regist. 57:22984-23005, 1992.

14. Scientific Committee for Food (SCF), Commission of the European Communities, Minutes of the 87th Meeting of SCF, Brussels, 1992.

15. JECFA (Joint FAO/WHO Expert Committee on Food Additives), Toxicological evaluation of certain food additives, 31st session, Geneva, 1987.

16. JECFA (Joint FAO/WHO Expert Committee on Food Additives), Evaluation of certain food additives and naturally occurring toxicants, 39th session, Geneva, 1995.

17. Elvig, S. G. and P. B. Pedersen. Safety evaluation of a glucanase preparation intended for use in food including a subchronic study in rats and mutagenicity studies. Reg. Toxicol. Pharm. 37:11-19, 2003.

18. Wine Institute. 2000. Per capita wine consumption by country. Wine Institute, The Voice for California Wine, Key Facts. Available from www.wineinstitute.com.

19. World Drinks, 2000. World drinks market growing by 3% a year. Italian Food and Beverage Technology, May, pp. 39-42.

Appendix 1

Product Sheet

novozymes®

Glucanex® 200 G

A unique beta-glucanase for winemaking

Description

Glucanex is a beta-glucanase preparation produced by submerged fermentation of a *Trichoderma harzianum* microorganism which has not been genetically modified. Glucanex has been especially developed in order to improve the clarification and filtration of wines produced from *Botrytis*-infected grapes. This improvement is the result of a selective enzymatic degradation of the glucan produced in the grapes by *Botrytis cinerea*. This polysaccharide is a beta-1,3-1,6-glucan.

Product properties

Activity

Glucanex glucanase activity ..... 200 BGXU/g

The product is a light-brown, soluble microgranulate without preservatives.

Activity determination

The beta-glucanase activity is measured on *Botrytis* glucan. A detailed description of Novozymes' analytical methods (BGXU: NNFCH 15) is available on request.

Packaging

Glucanex is available in 100 g tins packed in 1 kg boxes.

Food-grade status

The product complies with the following FCC and JECFA requirements: Antibiotic activity (Not detected), Mycotoxins (Not detected), Heavy metals (<30 ppm), Lead (< 5 ppm), Arsenic (< 3 ppm).

Application

Glucanex can be used in all cases where the aim is to improve clarification and filterability of wines made from botrytized grapes. Glucanex can be added to the wine any time between the first racking and the filtration.
It is recommended to treat the wines after the alcoholic fermentation in order to benefit from the higher temperature. The simultaneous use of Glucanex and bentonite should be avoided. Preferably, the glucanase treatment should be made prior to bentonite treatment.
The use of SO_2 up to 500 ppm has no influence on the enzyme activity.
Glucanex is added as a 10% solution directly into the tank. The temperature

during enzymatic treatment should preferably be above 12°C and treatment time at least 2 weeks.

Young white or rosé wine..... ... 1-3 g/hl or 38-114 g/1000 gal
(after alcoholic fermentation)

Solubility

The active enzyme components of Glucanex are readily soluble in water or must at all concentrations occurring in normal usage.

Safety

Enzymes are proteins and inhalation of dust or aerosols may induce sensitization and may cause allergic reactions in sensitized individuals. Some enzymes may irritate the skin, eyes and mucous membranes upon prolonged contact.
The product is developed to resist mechanical effects. However, excessive mechanical wear and tear or crushing may create dust. All spills, even small spills should be removed immediately. Use respiratory protection. Major spills should be carefully shovelled into plastic-lined containers. Small spills and remains of large spills should be removed by vacuum cleaning or flushing with water (avoid splashing). Vacuum cleaners and central vacuum systems should be equipped with HEPA filters. Wear suitable protective clothing, gloves and eye/face protection as prescribed on the warning label. Wash contaminated clothes.
Material Safety Data Sheets are supplied with all products. Further information describing how to handle the product safely is available on request.

Storage

Recommended storage conditions are 0-25°C in unbroken packaging, dry and protected from the sun. The product has been formulated for optimal stability. However, enzymes gradually lose activity over time. Extended storage or adverse conditions, including higher temperature or high humidity, may lead to a higher dosage requirement.

Novozymes Switzerland AG
Neumatt
4243 Dittingen
Switzerland
Tel. +41 61 7656111
Fax +41 61 7656333

Novozymes A/S
Krogshoejvej 36
2880 Bagsvaerd
Denmark
Tel. +45 8824 9999
Fax +45 8824 9998
info@novozymes.com
www.novozymes.com

Laws, regulations and third party rights may prevent customers from importing, processing, applying and/or reselling certain products in a given manner. It is the responsibility of the customers that their specific use of products from Novozymes does not infringe relevant laws and regulations and, furthermore, does not infringe patents or other third party rights.
The contents of this document are subject to change without further notice.

2001-11119-03 Feb 2002 © Novozymes A/S

Appendix 2

Pages 000022 - 000030 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

SUBMISSION END

000031

Reference List for Industry Submission, GRN 000149

Pages	*Author*	*Title*	*Publish Date*	*Publisher*	*BIB_Info*
000022 - 000030	Elvig, S. G. ; Pedersen, P.B.	Safety evaluation of a glucanase preparation intended for use in food including a subchronic study in rats and mutagenicity studies	2003	Regulatory Toxicology and Pharmacology	Volume 37, pgs 11-19

NA- Not applicable